Reversing HOXA9 Oncogene Activation by PI3K Inhibition: Epigenetic Mechanism and Prognostic Significance in Human Glioblastoma

Bruno M. Costa1,2, Justin S. Smith1, Ying Chen1, Justin Chen1, Heidi S. Phillips3, Kenneth D. Aldape4, Giuseppe Zardo1,5, Janice Nigro6, C. David James1, Anderson Cancer Center, Houston, Texas; 5Department of Cellular Rome, Italy; and6University of Bergen-Institute of Biomedicine, Bergen, Biotechnologies and Hematology, University of Rome-La Sapienza, Inc., South San Francisco, California;4Department of Pathology, M.D.

Abstract

HOXA genes encode critical transcriptional regulators of embryonic development that have been implicated in cancer. In this study, we documented functional relevance and mechanism of activation of HOXA9 in glioblastoma (GBM), the most common malignant brain tumor. Expression of HOXA genes was investigated using reverse transcription–PCR in primary gliomas and glioblastoma cell lines and was validated in two sets of expression array data. In a subset of GBM, HOXA9 genes are aberrantly activated within confined chromosomal domains. Transcriptional activation of the HOXA cluster was reversible by a phosphoinositide 3-kinase (PI3K) inhibitor through an epigenetic mechanism involving histone H3K27 trimethylation. Functional studies of HOXA9 showed its capacity to decrease apoptosis and increase cellular proliferation along with tumor necrosis factor–related apoptosis-including ligand resistance. Notably, aberrant expression of HOXA9 was independently predictive of shorter overall and progression-free survival in two GBM patient sets and improved survival prediction by MGMT promoter methylation. Thus, HOXA9 activation is a novel, independent, and negative prognostic marker in GBM that is reversible through a PI3K-associated epigenetic mechanism. Our findings suggest a transcriptional pathway through which PI3K activates oncogenic HOXA expression with implications for mTOR or PI3K targeted therapies.

Introduction

Glioblastoma (GBM) is the most common and malignant primary brain tumor in adults. With rare exception, the infiltrative nature of GBM precludes surgical cure and adjuvant therapies have achieved only modest benefit, with median survival remaining ~12 months (1). Specific molecular characteristics of GBM can be used to define prognostic subgroups (2–4). MGMT promoter methylation (5, 6) is an independent favorable prognostic factor for GBM patients (3), but this marker does not fully account for the variability in survival (3), and additional markers are needed to be used for the prognosis of individual patients.

Homeobox genes encode transcription factors important for antero-posterior patterning during embryogenesis. In humans, there are 39 Class I homeobox (HOX) genes found in four genomic clusters (HOXA at 7p15.3, HOXB at 17q21.3, HOXC at 12q13.3, and HOXD at 2q31). Their spatial and temporal colinear expression pattern is critical for body patterning during development (7, 8), and HOX genes have also critical postdevelopmental regulatory functions (9–12). Aberrant HOX gene expression initiates leukemias (13, 14), contributes to multiple types of solid tumors (15, 16), and has been detected in malignant astrocytomas (17). Furthermore, HOXA9 and MEIS1 may be part of a switch that regulates progenitor abundance by suppressing differentiation and maintaining self-renewal during myelopoiesis (18). Thus, among the 39 HOX genes, HOXA9 is of particular importance in leukemia and potentially other cancers.

In the present study, we identify a subgroup of GBMs with chromosomal domains of aberrant HOX gene activation, show a mechanism by which this domain of activation is reversible, define an association between HOXA9 expression and poor clinical outcome, and provide functional data on the effects of HOXA9 in cell proliferation and apoptosis.
Materials and Methods

Human tissues and glioma cell models. Human tissue samples were obtained from the Neurosurgery Tissue Banks at University of California, San Francisco (UCSF) and M.D. Anderson Cancer Center (MDA) and would have been otherwise discarded. Tumor samples were examined by a neuropathologist to ensure that >90% of the tissue represented tumor and classified according to current WHO guidelines (19). All samples were collected with informed consent, and investigations using human tissues and clinical data were approved by the institutional review boards of UCSF and MDA. Initial exploratory survival analysis was performed on a set of nine GBMs from UCSF. Validation of a trend between HOXA9 expression and poor survival was performed using two sets of expression array data (Affymetrix U133 arrays or Affymetrix U95Av2 arrays), one set consisting of 37 GBM samples from UCSF (20) and a second set composed of 63 GBMs from MDA (ref. 21; Gene Expression Omnibus accession number GSE4271). Combined assessment of MDA (ref. 21; Gene Expression Omnibus accession number from UCSF (20) and a second set composed of 63 GBMs from MDA (ref. 21; Gene Expression Omnibus accession number GSE4271). Combined assessment of MDA (ref. 21; Gene Expression Omnibus accession number GSE4271).

In vitro analyses. RNA isolation, cDNA synthesis, reverse transcription–PCR (RT-PCR), quantitative real-time PCR (qPCR), and chromatin immunoprecipitation (ChiP) analyses were performed by standard techniques in cell lines (Supplementary Materials and Methods). DNA isolation, bisulfite treatment, and analyses of MGMT promoter methylation in primary tumor tissues from UCSF were performed as described previously (3, 25) and are detailed in Supplementary Materials and Methods. A172 cells and neurospheres were treated with a phosphoinositide 3-kinase (PI3K) inhibitor (LY294002) and rapamycin to investigate the mechanism regulating HOXA4 gene expression in GBM. Overexpression of HOXA9 by retroviral infection and siRNA-mediated silencing of HOXA9 were used to investigate the functional relevance of HOXA9 expression, which was assessed by cell proliferation and apoptosis analyses (Supplementary Materials and Methods).

Statistical analyses. The Kaplan-Meier method was used to estimate overall survival (OS) and progression-free survival (PFS), where OS was measured from the time of surgical resection to death or the last date when the patient was known to be alive, and PFS was defined as the time from surgical resection to the time of shown tumor growth on follow-up imaging or death, if death occurred before documented progression. Multivariate survival analyses by Cox proportional hazard models (backward selection) were performed to adjust for the effects of patient age and Karnofsky performance status (KPS). A P value of <0.05 was considered significant.

The two-sided Student’s t test was used to assess statistical differences in qPCR data and in vitro cell death and apoptosis experiments. A repeated measures ANOVA was used to assess differences in the cell proliferation curves.

To identify samples that express a given gene and assign the confidence to each sample, we used model-based clustering to identify the mixture components. Analysis of gene expression microarray data, generation of heat maps for the HOX clusters, and investigation of correlations between expression of HOXA9 and other transcripts are detailed in Supplementary Materials and Methods.

Results

HOX genes are predominantly activated in high-grade astrocytoma. Initially, we assessed HOXA gene expression in normal brain tissue, primary diffuse glioma tissue, and GBM-derived cell lines using RT-PCR. Most HOXA genes were not expressed in fetal and adult nontumoral brain tissues (Fig. 1A). Similarly, of eight primary grade 2 and grade 3 gliomas, HOXA4 gene expression was evident in only two cases (Fig. 1A). In contrast, all primary GBMs and cell lines showed expression of multiple HOXA4 genes (Fig. 1A). Supplementary Fig. S1 shows representative RT-PCR analyses of HOX4 gene expression in human astrocytoma samples, exemplifying tumors that were positive or negative for expression of specific HOXA genes. Collectively, these data suggest that aberrant HOXA4 gene expression is a common feature of the most malignant gliomas, GBM, and infrequent in lower-grade gliomas.

HOXA9 was the only HOX gene whose expression showed a trend to associate with shorter survival of these nine GBM patients (median survival of 9 versus 53 weeks, P = 0.06). Supplementary Fig. S1 (B and C) suggests HOXA9 expression in GBMs is not simply a reflection of the expression pattern of the tumor cell(s) of origin nor a consequence of therapeutic agents but rather represents an aberrant gene activation event associated with tumor progression.

A confined chromosomal domain of transcriptional activation encompasses HOXA expression in GBM. We next analyzed expression of HOXA genes in two independent validation sets of expression array data, one set of 37 GBMs from UCSF (20) and a second set of 63 GBMs from MDA (21). To investigate whether aberrant HOXA activation was an isolated event, or part of a larger chromosomal mechanism, we created heat maps for the HOXA cluster and surrounding genes within 1 Mb in each direction (Fig. 1B). In both sets, the HOXA cluster showed a distinct chromosomal domain of activation such that many of the HOX genes were mostly silent (Fig. 1B). Similar domains of transcriptional activation were also present in the HOXB, HOXC, and HOXD clusters (Supplementary Fig. S2 and S3), suggesting a common, coordinated activation of these domains.

Heat map analyses reflecting the expression pattern across all four HOX clusters in UCSF and MDA tumor sets analyzed
by expression arrays (Supplementary Fig. S4) revealed that (a) a subset of GBMs shows widespread activation of HOX genes, (b) the pattern of HOX activation is aberrant and does not resemble the coordinated colinear expression observed during normal embryonic development, (c) HOXA9 substantially contributes to the clustering into two sample groups in both data sets. To quantify which of the HOX genes account for the clustering, the fold change between the two groups for each gene was computed using the median value of the genes in each group (Supplementary Table S1). The HOX genes with statistically significant different expression between the two heat map cluster groups in both the UCSF and MDA tumors are HOXA1-A5, HOX7, HOXA9, HOXA10, HOXB7, and HOXC6.

We used the ONCOMINE database to investigate additional GBM expression array data sets for activation of the HOXA domain (26). A subset of GBM patients showed a similar profile of activation of HOX genes in a study by Sun and colleagues (27). Expression of most HOX genes, with the exception of HOXA6 and HOXA13, showed a high positive correlation with HOXA9 expression (Supplementary Table S2). HOXA11, which did not show a statistically significant positive correlation with HOXA9 expression in our tumor sets, also showed a substantially lower correlation coefficient in the samples from Sun and colleagues (27). Because HOXA6 and HOXA13 do not correlate with HOXA9 expression in all three tested tumor sets, a mechanism of chromosomal amplification encompassing the entire HOXA locus may not adequately explain the presence of such transcriptionally active domains. Furthermore, we confirmed in the UCSF set that aberrant HOX gene activation may be enhanced by, but is not reliant on, increased chromosome 7p15.3 copy number (P = 0.5, data not shown).

**Inhibition of the PI3K pathway reverses aberrant transcrip
tional activation of the HOXA cluster.** In HeLa cells, the PI3K signaling pathway can regulate EZH2 activity (28), a key component of the polycomb repressor complex 2 that promotes gene silencing by trimethylation of histone H3 lysine 27 (H3K27-met) on target genes. AKT-mediated phosphorylation of EZH2 inhibits its histone methyltransferase activity, resulting in gene reactivation. Given that HOXA genes are known targets of EZH2-mediated methylation in some normal tissues (29) and that PTEN, a regulator of the PI3K pathway, are currently in clinical trials for primary GBM xenografts (32), a subset of GBMs shows widespread activation of HOX genes within domains of activation in primary GBMs, we hypothesized that HOX genes in GBM are activated by a shared mechanism of transcriptional regulation. To address this question, the entire HOXA cluster was tested for suppression by LY294002 treatment of A172 cells. Expression of most HOX genes was suppressed (range, 39–74%) after treatment with LY294002, except for HOXA6 and HOXA13 (Fig. 2A); these two HOX genes also did not correlate with HOXA9 expression in our primary tumor sets (Supplementary Table S1) or in the cohort studied by Sun and colleagues (27). These data suggest that HOXA9 expression is reversibly regulatable by the PI3K pathway in glioma cells and further suggest that this regulation extends throughout most of the 100+ kb chromosomal domain containing the HOXA cluster.

To further support our data implicating the PI3K pathway as a critical regulatory mechanism of HOX genes expression, we next searched the Connectivity Map data set for drugs that induce gene expression signatures involving HOXA9-associated genes (33). This analysis revealed common PI3K inhibitors, including LY294002 and Wortmannin, as top hits significantly associated with the HOXA9-derived gene expression signature (Supplementary Table S3 and Supplementary Results: Connectivity Map analyses reveal the PI3K pathway as a key regulator of HOX genes expression). Additionally, in primary GBM xenografts derived from 18 patients, aberrant activation of HOX9 was significantly associated with PTEN gene inactivation [PTEN mutations and homozygous deletion were previously examined by sequence analysis and multiplex PCR analysis, respectively (22, 24)], further supporting the relevance of PI3K signaling for the regulation of HOXA expression (Supplementary Table S4 and Supplementary Results: aberrant expression of HOXA9 is associated with PTEN gene inactivation in primary GBM xenografts).

Therapeutic agents that inhibit mTOR, a downstream mediator of the PI3K pathway, are currently in clinical trials for GBM. To determine whether mTOR is a critical regulator of HOX4 gene expression, A172 cells were treated with rapamycin, an inhibitor of mTOR activity, and the expression levels of the HOX4 genes were assessed by RT-PCR. Rapamycin treatment resulted in a more modest inhibition of HOX4 transcripts levels (Fig. 2B), suggesting that the mechanism by which the PI3K pathway regulates HOX4 genes expression is primarily independent of mTOR. HOX9 protein levels were also unaffected by rapamycin treatment as indicated by immunoblotting analysis (Supplementary Fig. S5C).

To determine whether the PI3K-mediated regulation of HOX4 genes is also observed in other GBM cell lines, we tested how LY294002 treatment affected HOX9 levels by qPCR in two sublines of a primary GBM grown in neurosphere conditions, both of which presented detectable levels of endogenous HOXA9. Similarly to the observations in A172 cells, the inhibition of PI3K in these neurospheres also repressed HOX9 mRNA levels (Supplementary Fig. S5D), whereas the relationship between rapamycin and HOX9 expression in the very small number of neurosphere lines is not as consistent (Supplementary Fig. S5E).
PI3K-mediated regulation of HOXA9 gene expression occurs through reversible epigenetic histone modifications.

To elucidate the downstream effectors of PI3K-mediated regulation of HOXA gene expression and considering that AKT mediates EZH2 histone methyltransferase activity in HeLa cells, we assessed whether the regulatory role of the PI3K pathway on HOXA9 expression could be linked to histone modifications. ChIP analysis was performed on A172 cells.
Figure 2. The PI3K pathway regulates HOXA cluster gene transcription in GBM through reversible epigenetic histone modifications, independently of mTOR. A, expression levels of genes in the HOXA cluster by RT-PCR after treatment of A172 cells with the PI3K inhibitor LY294002. β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as control genes. Values are ratios of expression levels in treated/untreated A172 cells for each HOXA gene. Expression of most HOXA genes, except for HOXA6 and HOXA13, decreased after PI3K inhibition. HOXA1 and HOXA9 expression was assessed for each of two alternative splicing forms (i and ii). The results are representative of three independent experiments. B, expression levels of HOXA genes after treatment with rapamycin, a specific mTOR inhibitor. Data are presented similarly to A. The results are representative of three independent experiments. C, ChIP analysis of the HOXA9 5' region after LY294002-mediated inhibition of PI3K. Top, diagram indicating the locations of the primer sets used for ChIP analysis. Bottom, PCR products for the three regions after ChIP analysis in A172 cells treated with LY294002 (+) or vehicle (−). The input products reflect PCR on DNA not selected by immunoprecipitation. The IgG reaction serves as a negative control. The results are representative of three independent experiments.
that were either untreated or treated with LY294002 for 24 hours. A172 cells treated with LY294002 showed reduced expression of HOXA9, as well as reduced H3K4 trimethylation (a mark of active chromatin) and concomitant increased H3K27 trimethylation (a mark of repressive chromatin) in three different regions near the HOXA9 promoter (Fig. 2C). When LY294002-treated cells were incubated for an additional 48 hours in fresh media without drug, HOXA9 expression, H3K27 trimethylation, and H3K4 trimethylation returned to pretreatment levels (data not shown). Collectively, these data indicate that the regulatory role of the PI3K pathway on HOXA9 expression is linked to epigenetic histone modifications that seem to be fully and expediently reversible in GBM cells.

**HOXA9 increases cell proliferation and inhibits apoptosis.** To test the functional consequences of HOXA9 overexpression in vitro, a GBM cell line (U87-MG) and immortalized human astrocytes (hTERT/E6/E7) stably overexpressing HOXA9 were established by retroviral infection and compared with their counterparts infected with control vector, which do not overexpress HOXA9 (Fig. 3A). The use of these two GBM models allowed us to test the effect of HOXA9 overexpression in tumoral (U87-MG) and immortalized but nontumoral (hTERT/E6/E7) backgrounds. Expression of exogenous HOXA9 was associated with a modest but consistent increase in proliferation of hTERT/E6/E7 cells (Fig. 3B; \( P < 0.001 \)) and a decrease in their spontaneous apoptosis (Fig. 3C; \( P = 0.032 \)) compared with the parental cells. U87-MG cells stably expressing exogenous HOXA9 consistently showed a modest increase in proliferation, but only when the cells became more confluent (Fig. 3B; \( P < 0.001 \)). HOXA9 expression in U87-MG cells also decreased tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)–induced apoptosis (Fig. 3C; \( P = 0.009 \)). These data suggest that HOXA9 serves an antiapoptotic role in immortalized human astrocytes and GBM cells, which may affect cell number/proliferation. Conversely, the inhibition of endogenous HOXA9 expression in A172 cells with siRNA (Fig. 3D) resulted in increased spontaneous apoptosis.

![Image](https://cancerres.aacrjournals.org/doifigure/10.1158/0008-5472.CAN-09-2189-fig3a.png)

**Figure 3.** Expression of HOXA9 influences cell proliferation and apoptosis. A, immortalized human astrocytes (hTERT/E6/E7) and a GBM-derived cell line (U87-MG) were retrovirally infected with a MSCV-HOXA9 construct or control vector and tested for HOXA9 expression by RT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for equal loading control. Successful expression of exogenous HOXA9 was detected for both cell types. The two PCR bands for HOXA9 derive from amplification of specific alternative splicing isoforms. B, proliferation curves of hTERT/E6/E7 and U87-MG cells after retroviral infections. Results are representative of three independent experiments, and each time point was counted in duplicate (data points represent mean ± SD). Repeated measures ANOVA showed statistically significant differences between control cells and cells overexpressing HOXA9 (hTERT/E6/E7/Control versus hTERT/E6/E7/HOXA9, \( F_{(1,18)} = 92.7, P < 0.001 \); U87-MG/Control versus U87-MG/HOXA9, \( F_{(1,18)} = 19.7, P < 0.001 \)). C, apoptosis analysis by Annexin V–FITC and propidium iodide staining followed by flow cytometry. Results are representative of three independent experiments (averages ± SDs), each showing a similar effect relative to controls. *, \( P < 0.05 \), two-sided Student’s \( t \) test \( (P = 0.032 \) for hTERT/E6/E7/Control versus hTERT/E6/E7/HOXA9; \( P = 0.036 \) for spontaneous U87-MG/Control versus TRAIL-treated U87-MG/Control; \( P = 0.009 \) for TRAIL-treated U87-MG/Control versus TRAIL-treated U87-MG/HOXA9). D, left, qPCR analysis of HOXA9 expression levels after treatment with siHOXA9 or siControl for various time points. Expression levels were normalized to hGUS. *, \( P < 0.05 \), two-sided Student’s \( t \) test \( (P = 0.004 \) for 24 h; \( P = 0.001 \) for 48 h). Right, apoptosis analysis by Annexin V–FITC and propidium iodide staining followed by flow cytometry 48 h after siRNA treatments. The results are representative of three independent experiments (averages ± SDs), each showing a similar effect relative to controls. *, \( P = 0.026 \), two-sided Student’s \( t \) test.
Further supporting an antiapoptotic role of HOXA9 in GBM cells, HOXA9 expression is associated with shorter OS and PFS in GBM patients. Based on the association of HOXA9 with poor prognosis in the initial test set of nine GBM patients, we then performed survival analysis using the UCSF and MDA sets of expression array data. To validate the expression array data, RT-PCR for HOXA9 was performed on the subsets of cases with the highest and lowest HOXA9 expression array values, and the results were concordant (Fig. 4A and B, insets). In the UCSF cohort, GBM patients whose tumors expressed HOXA9 (5 of 37, 14%) had a significantly shorter OS [median, 22 weeks; 95% confidence interval (CI), 5–38 weeks] compared with those whose tumors lacked HOXA9 expression (median, 68 weeks; CI, 36–100 weeks; P < 0.0001; Fig. 4A). Similarly, in the MDA set, patients whose tumors expressed HOXA9 (14 of 63, 22%) also had a shorter median OS (56 weeks; CI, 10–102 weeks) than those whose tumors lacked HOXA9 expression (median OS, 91 weeks; CI, 46–136 weeks; P = 0.03; Fig. 4B). Patients whose tumors expressed HOXA9 also had significantly shorter PFS. Median PFS was 4 weeks (CI = 4–5 weeks) and 30 weeks (CI = 22–38 weeks) in the UCSF set and 46 weeks (CI = 17–76 weeks) and 77 weeks (CI = 22–132 weeks) in the MDA set for patients whose tumors did and did not express HOXA9, respectively (P < 0.0001, Fig. 4C, UCSF set; P = 0.03, Fig. 4D, MDA set). These associations remained statistically significant after adjusting for the effects of patient age and KPS in the UCSF set (P = 0.006 for OS, P = 0.01 for PFS). KPS data were not available for the MDA cohort.

**HOXA9 expression status improves MGMT-based survival prediction in GBM patients.** MGMT promoter methylation in tumor tissue is currently the most powerful molecular prognostic indicator of favorable prognosis in GBM.
GBM (3). However, a significant subset of patients whose tumors show MGMT promoter methylation do not experience survival benefit. To contrast the prognostic significance of HOX9 expression with this biomarker standard, we performed methylation-specific PCR analysis on DNA available from 34 of the 37 UCSF GBMs previously analyzed by expression array (Fig. 1B) and from the original nine GBMs analyzed by RT-PCR (Fig. 1A). No DNA was available from the MDA set to perform methylation-specific PCR. MGMT promoter methylation was identified in 24 of the 43 cases (56%; Supplementary Fig. S7); as expected (3), these patients showed trends toward longer OS ($P = 0.1$; Fig. 5A) and PFS ($P = 0.06$; Supplementary Fig. S6A) compared with those whose tumors lacked MGMT methylation. HOX9 and MGMT in combination showed statistically significant associations with OS and PFS. Patients whose tumors showed HOX9 expression and/or lack of MGMT promoter methylation had significantly shorter OS (median, 31 weeks; CI, 3–60 weeks) and PFS (median, 13 weeks; CI, 5–20 weeks) compared with patients whose tumors lacked HOX9 expression and had MGMT promoter methylation (median, 82 weeks; CI, 41–123 weeks for OS; $P = 0.002$; Fig. 5B; median, 33 weeks; CI, 22–44 weeks for PFS; $P = 0.001$; Supplementary Fig. S6B). These associations remained statistically significant after adjusting for the effects of patient age and KPS ($P = 0.02$ for OS, $P = 0.03$ for PFS). Expression of HOX9 was identified in five (21%) of the tumors with a methylated MGMT promoter and, among these patients, was associated with a significantly shorter OS (median survival of 9 versus 98 weeks, $P < 0.0001$; Fig. 5C) and shorter PFS (median PFS of 4 versus 33 weeks, $P < 0.0001$; Supplementary Fig. S6C).

## Discussion

Multiple HOX genes have been shown to be overexpressed in GBM cell lines and primary astrocytomas (17), suggesting a role for these genes in gliomagenesis. However, the mechanisms underlying HOX activation, in addition to their functional relevance in GBM cells, have not been explored. Our results define PI3K-regulatable chromosomal domains of transcriptional activation as a means of aberrant gene expression in cancer. This adds to a growing perspective on chromosomal domains of epigenetic silencing in normal and malignant cells (34–36). The domains of HOX transcriptional activation we discovered have notable features: (a) they involve an important set of genes, which have critical roles in embryonic development, normal adult tissue function, and oncogenesis; (b) they are mediated through reversible epigenetic histone modifications that are regulatable through the PI3K pathway, a pathway known to be frequently altered in many cancers (37); and (c) they include aberrant expression of the oncogenic HOX9 (13, 38), associated with histologic malignant progression, shorter time to tumor progression and shorter OS in GBM patients. Our functional data provide further support for an oncogenic role of HOX9 in GBM. Furthermore, a recent study suggested HOX genes may be part of a glioma stem cell signature with prognostic significance in GBM patients treated with concomitant chemoradiotherapy (16). Our data identify PI3K pathway dysfunction as a potential driver of this signature and suggest the exciting possibility that PI3K inhibitors may allow therapeutic reversal of the core element of this cancer stem cell signature. We implicate the PI3K pathway in the generation of aberrant HOX domains of activation in GBM. Activation of the PI3K pathway in glioma is significantly associated with increasing tumor grade, decreased apoptosis, and adverse clinical outcome (37). In addition, PTEN, a central regulator of the PI3K pathway, is frequently altered in GBM and, when altered in lower-grade gliomas, portends a dismal prognosis (1). Although multiple downstream mediators of the PI3K pathway have been described and much focus is placed on translational effects of PI3K pathway dysregulation, we propose that the pathway arm involving EZH2, a central member of the polycomb repressive complexes with intrinsic
histone methyltransferase activity (39), mediates the PI3K-dependent transcriptional regulation of HOXA9 and potentially the whole HOXA cluster and many other genes. Akt-mediated phosphorylation of EZH2 suppresses trimethylation of lysine 27 in histone H3 (H3K27) by interfering with the ability of the EZH2 complex to interact with histone H3, leading to deregression of silenced genes (28). EZH2 can increase cancer cell proliferation when overexpressed (40), is associated with metastasis in prostate cancer (41), and is expressed at significantly higher levels in GBM tissue versus noncancerous brain tissue (26, 27) and low-grade gliomas (26, 42). Furthermore, EZH2 itself was among the genes that showed coexpression with HOXA9 based on expression array data from both UCSF and MDA tumor sets. However, the activity of overexpressed EZH2 may well be inhibited by Akt-mediated phosphorylation. Regulation of HOXA expression is even more complex, involving other histone marks, noncoding RNA, and microRNA-mediated regulation depending on the cellular context (43). Because epigenetic mechanisms are interdependent, PI3K-driven changes in H3K27 methylation may influence additional histone modifications, either directly or indirectly. In fact, the PI3K pathway has been linked to other specific histone modifications, such as H3K9 demethylation (44), acetylation of H3K9 and H3K18 (45), and deacetylation of H3K14 (45). Thus, therapeutic agents targeting components of the PI3K pathway may indirectly affect the histone marks of tumor cells. We hypothesize that particular profiles of histone modifications, in conjunction with the status of the PI3K pathway, may influence therapeutic decisions in the future. Indeed, whereas the histone profile of malignant cells is not currently used to direct therapeutic decisions, histone modifications are strongly correlated with clinical outcome of tumor patients (46–50).

Considerable focus has been placed on developing chemotherapeutic agents to inhibit mTOR, a downstream effector of the PI3K pathway that is an important regulator of cell growth and metabolism (51). Whereas these agents are expected to suppress the growth effects of the mTOR pathway, it is less clear whether mTOR is a factor regulating the expression of HOXA genes in GBM. If these domains of transcriptional activation contribute to tumor growth in vivo, mTOR inhibitors alone may be suboptimal for improving the outcome in a subset of GBM patients. PI-103, a recently reported agent that dually inhibits PI3K and mTOR in glioma cells (52) or similar agents may prove more efficacious.

**References**


**MGMT** promoter methylation is a favorable prognostic indicator in GBM but is limited in its clinical applicability to individual patients. HOXA9 expression is a marker of poor prognosis, independent of MGMT promoter methylation status, and identifies a subset of the patients who, despite apparently favorable MGMT promoter methylation, have poor outcomes. Thus, the combined assessment of HOXA9 expression and MGMT methylation status in GBM may prove to be a promising effective prognostic tool. The grade specificity of HOXA9 expression according to histology and the association with poor outcome among GBM patients suggest that it may be useful as part of a molecular-based classification of gliomas. The prognostic relevance of HOXA9 expression in new experimental therapies warrants further investigation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank Drs. Corey Largmann and H. Jeffrey Lawrence for helpful discussion regarding HOXA9 and for sharing retroviral constructs, Dr. Russell Pieper for supplying hTERT/E6/E7 cells, Dr. Amith Paner for assistance with molecular techniques, Dr. Susan Chang for helpful discussion regarding the clinical management of GBM patients, and Drs. Scott Vandenberg and Andrew Bollen for neuropathological assessment of tumors used in this study.

**Grant Support**

NIH grants NIH CA094971 (J.F. Costello) and NIH/NCI F32 CA113039-01 (J.S. Smith); Karen Osney Brownstein Endowed Chair (J.F. Costello); UC Discovery grant Bio05-10501 (J.F. Costello and H.S. Phillips); Portuguese Science and Technology Foundation SFRH/BD/15258/2004 (B.M. Costa); and Luso-American Development Foundation, Portugal 186/06 (B.M. Costa).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 6/17/09; revised 11/4/09; accepted 11/5/09; published OnlineFirst 1/12/10.


Correction: Online Publication Dates for Cancer Research April 15, 2010 Articles

The following articles in the April 15, 2010 issue of Cancer Research were published with an online publication date of April 6, 2010 listed, but were actually published online on April 13, 2010:


Correction


Dudka AA, Sweet SMM, Heath JK. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor receptor is activated by receptor amplification. Cancer Res 2010;70:3391–401. Published OnlineFirst April 13, 2010. doi:10.1158/0008-5472.CAN-09-3033.


Published OnlineFirst 05/11/2010.
©2010 American Association for Cancer Research.
doi: 10.1158/0008-5472.CAN-10-1347
Reversing *HOXA9* Oncogene Activation by PI3K Inhibition: Epigenetic Mechanism and Prognostic Significance in Human Glioblastoma

Bruno M. Costa, Justin S. Smith, Ying Chen, et al.

*Cancer Res* 2010;70:453-462. Published OnlineFirst January 12, 2010.

**Updated version**
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-2189

**Supplementary Material**
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2010/01/11/0008-5472.CAN-09-2189.DC1

**Cited articles**
This article cites 52 articles, 23 of which you can access for free at:
http://cancerres.aacrjournals.org/content/70/2/453.full.html#ref-list-1

**Citing articles**
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/70/2/453.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.